

**AMENDMENTS TO THE SPECIFICATION:**

Please replace the paragraph beginning at page 28, line 24, with the following rewritten paragraph:

The feasibility of the immunogen production approach of the present invention has been shown using ~~BiaCore~~ BIACORE 3000 technology. Figure 4 shows that CD4, not CD8, can be bound to gp120. Figures 5, 6 show the differences in interaction of gp120 with the envelop of primary isolates and lab-adapted HIV strains. Figure 7 shows that vesicles from chimeric hamster ovary cells transfected with HIV-IIIB gp160 and that these vesicles, present on a ~~BiaCore~~ BIACORE L1 Chip, show stabilized binding of soluble CD4 but not CD8.

Please replace the paragraph beginning at page 30, line 25, with the following rewritten paragraph:

Synthetic peptides were synthesized (SynPep Corporation, Dublin, CA), and purified by reverse phase HPLC. Peptides used in this study had greater than 95% purity as determined by HPLC, and confirmed to be correct by mass spectrometry. The CCR5-D1 (MDYQVSSPIYDINYYTSEPCQKINVKQIAAR) (SEQ ID NO:1), peptide was derived from the N-terminus of human CCR5 (Bieniasz et al, EMBO Journal 16:2599-2609 (1997)). Gp41 peptides DP-178  
YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF (SEQ ID NO:2) (Wild et al,

Proc. Natl. Acad. Sci. USA 91:12676-12680 (1994)), T-649

WMEWDREINNYTSLIHSLIEESQNQQEKNEQELLEL (SEQ ID NO:3) (Rimsky et al, J. Virol. 72:986-993 (1998)), and T649-Q26L

(WMEWDREINNYTSLIHSLIEESQNQLEKNEQELLEL) (SEQ ID NO:4) (Shu et al, Biochemistry 39:1634-1642 (2000)) were derived from HIV-1 envelope gp41 from HIV 89.6 (Collmann et al, J. Virol. 66:7517-7521 (1992)). As a control for HR-2 peptide binding, a scrambled sequence DP178 peptide was made as well.

Please replace the paragraph beginning at page 31, line 17, with the following rewritten paragraph:

SPR biosensor measurements were determined on a ~~BIAcore~~ BIACORE 3000 (~~BIAcore~~ BIACORE Inc., Uppsala, Sweden) instrument. HIV envelope proteins (gp120, gp140, gp160) and sCD4 were diluted to 100-300 mg/ml in 10mM Na-Acetate buffer, pH 4.5 and directly immobilized to a CM5 sensor chip using standard amine coupling protocol for protein immobilization (Alam et al, Nature 381:616-620 (1996)). Binding of proteins and peptides was monitored in real-time at 25°C and with a continuous flow of PBS, pH 7.4 at 5-20ml/min. Analyte (proteins and peptides) were removed and the sensor surfaces were regenerated following each cycle of binding by single or duplicate 5-10 ml pulses of regeneration solution (10 mM glycine-HCl, pH 2.5 or 10mM NaOH).

Please replace the paragraph beginning at page 32, line 5, with the following rewritten paragraph:

All analyses were performed using the non-linear fit method of O'Shannessy et al. (O'Shannessy et al, Anal. Biochem. 205:132-136 (1992)) and the BIAevaluation 3.0 software (BIAcore BIACORE Inc). Rate and equilibrium constants were derived from curve fitting to the Langmuir equation for a simple bimolecular interaction ( $A + B = AB$ ).

Please replace the paragraph beginning at page 42, line 22, with the following rewritten paragraph:

*Neutralizing Epitopes on HIV 89.6 gp140 Before and After Ligation with sCD4.* The 2F5 (anti-gp41, ELDKWAS (SEQ ID NO:5)) (Muster et al, J. Virol. 67:6642-6647 (1993)), mab neutralizes HIV primary isolates. Prior to ligation of cleaved 89.6 gp140 with sCD4, it was found that the 2F5 gp41 epitope was exposed. Following sCD4 ligation, the 17b CCR5 binding site epitope (2-4) was upregulated and the 2F5 epitope continued to be expressed.

Please replace the paragraph beginning at page 44, line 7, with the following rewritten paragraph:

*HR-2 Peptides.* Synthetic peptides were synthesized (SynPep, Inc., Dublin, CA), and purified by reverse phase HPLC. Peptides used in this study had greater than 95% purity as determined by HPLC, and confirmed to be correct by mass spectrometry. gp41 peptides DP178, YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF (SEQ ID NO:2) (Wild et al, Proc. Natl. Acad. Sci. USA 19:12676-12680 (1994)), and T649-Q26L,

WMEWDREINNYTSLIHSLIEESQNQLEKNEQELLEL (SEQ ID NO:4) (Rimsky et al, J. Virol. 72:986-993 (1998), Shu et al, Biochemistry 39:1634-1642 (2000)) were derived from HIV-1 envelope gp41 from HIV 89.6 (Collman et al, J. Virol. 66:7517-7521 (1992)). As a control for HR-2 peptide binding, a scrambled sequence DP178 peptide was made. For immunoprecipitations and select SPR experiments, biotinylated DP178 and DP178 scrambled peptides were synthesized (SynPep, Inc.).

Please replace the paragraph beginning at page 44, line 24, with the following rewritten paragraph:

*Surface Plasmon Resonance Biosensor Measurements.* SPR biosensor measurements were determined on a ~~BIAcore~~ BIACORE 3000 (~~BIAcore~~ BIACORE Inc., Uppsala, Sweden) instrument. Anti-gp120 mab (T8) or sCD4 (100-300 µg/ml) in 10mM Na-Acetate buffer, pH 4.5 were directly immobilized to a CM5 sensor chip using a standard amine coupling protocol for protein immobilization (Alam et al, Nature 381:616-620 (1996)). A blank in-line reference surface (activated and de-activated for amine coupling) was used to subtract non-specific or bulk responses. Binding of proteins and peptides (biotinylated or free DP178, T649Q26L, DP178-scrambled) was monitored in real-time at 25°C with a continuous flow of PBS (150 mM NaCl, 0.005% surfactant p20), pH 7.4 at 10-30µl/min. Analyte (proteins and peptides) were removed and the sensor surfaces were regenerated following each cycle of binding by single or duplicate 5-10 µl pulses of regeneration solution (10 mM glycine-HCl, pH 2.5 or 10mM NaOH).

Please replace the paragraph beginning at page 45, line 15, with the following rewritten paragraph:

All analyses were performed using the non-linear fit method of O'Shannessy et al. (O'Shannessy et al, Anal. Biochem. 205:132-136 (1992)) and the BIAevaluation 3.0 software (BIAcore BIACORE Inc). Rate and equilibrium constants were derived from curve fitting to the Langmuir equation for a simple bimolecular interaction ( $A + B = AB$ ).

Please replace the paragraph beginning at page 49, line 25, with the following rewritten paragraph:

*Peptides.* Peptides were synthesized (SynPep, Inc., Dublin, CA), and purified by reverse phase HPLC. Peptides used in this study had greater than 95% purity as determined by HPLC, and confirmed to be correct by mass spectrometry. HR-2 gp41 peptide DP178, YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF (SEQ ID NO:2), and DP107, NNLLRAIEAQQHLLQLTVWGIKQLQARILAVEERYLKDQ (SEQ ID NO:6) were derived from HIV-1 89.6 envelope gp41 HR-2 and HR-1 regions respectively (Collman et al, J. Virol. 66(12):7517-7521 (1992), Wild, Proc. Natl. Acad. Sci. 91:9770-9774 (1994)). As a control for HR-2 peptide binding, randomly scrambled sequences of DP178 (scrDP178) and DP107 (scrDP107) peptides were also made. For precipitations and surface plasmon resonance (SPR) experiments using the streptavidin chip, biotinylated DP178 and scrDP178 peptides were synthesized (SynPep, Inc.). The

following C4 and V3 peptides were used in the peptide blocking experiment- V3<sub>89.6P</sub> – TRPNNNTRERLSIGPGRAFYARR (SEQ ID NO:7); C4- IKQIINMWQKVGKAMYAPPIS (SEQ ID NO:8); C4-V3<sub>MN</sub> – KQIINMWQEVGKAMYACTRPNYNKRKRRIHIGPGRAFYTTK (SEQ ID 9); and C4-V3<sub>89.6P</sub> – KQIINMWQEVGKAMYATRPNNNTRERLSIGPGRAFYARR (SEQ ID NO:10). A scrambled amino acid version of the V3 component of C4-V3 (C4-scrV3) was also synthesized as a control peptide.

Please replace the paragraph beginning at page 50, line 22, with the following rewritten paragraph:

*Surface Plasmon Resonance Biosensor Measurements.* SPR biosensor measurements were determined on a BIAcore BIACORE 3000 (BIAcore BIACORE Inc., Uppsala, Sweden) instrument and data analysis was performed using BIAevaluation 3.0 software (BIAcore BIACORE Inc). For the “capture assay”, anti-gp120 mAb (T8, A32) or sCD4 (100-300 µg/ml) in 10mM Na-Acetate buffer, pH 4.5 were directly immobilized to a CM5 sensor chip using a standard amine coupling protocol for protein immobilization (Alam et al, Nature 381:616-620 (1996)). A blank in-line reference surface (activated and de-activated for amine coupling) was used to subtract non-specific or bulk responses. Binding of proteins and peptides (biotinylated or free DP178/T-20, scrDP178) was monitored at 25°C with a continuous flow of PBS (150 mM NaCl, 0.005% surfactant p20), pH 7.4 at 10-30µl/min. Analyte (proteins and peptides) were

removed and the sensor surfaces were regenerated by single or duplicate 5-10  $\mu$ l pulses of regeneration solution (10 mM glycine-HCl, pH 2.5 or 10mM NaOH). For determination of HR-2 peptide specific binding, it was critical to use freshly prepared peptides prior to each experiment in order to minimize background binding to CD4 surfaces. Additionally, non-specific binding of HR-2 peptides to capture surfaces (CD4 or mAb T8) was subtracted to determine specific binding of HR-2 peptides to gp120 envelope proteins (Fig 27). Antibody blocking experiments were performed by mixing gp120 with excess (3-5 fold) of mAbs and pre-incubating at RT for 20 min. These mixtures were then injected for binding studies as described above.

Please replace the paragraph beginning at page 53, line 2, with the following rewritten paragraph:

*Inducible binding of HR-2 peptide to soluble HIV-1 Env gp120.* In initial experiments, when 89.6 gp120 was covalently immobilized on a ~~BIAcore~~ BIACORE CM5 sensor chip, it was observed that in comparison to scrambled DP178 (scrDP178) and in the presence of sCD4, the HR-2 peptide DP178 bound specifically to 89.6 Env gp120 proteins. These data suggested that there might be an HR-2 binding site on Env gp120. However, this was not a preferred protocol since random covalent coupling could lead to heterogeneity in the immobilized envelope protein and may also cause alterations in protein conformation. In addition, since the goal was to determine whether the gp120 HR-2 binding site was inducible by sCD4 or A32 mAb, the decision was made not to

employ a binding assay that required direct immobilization of Env proteins. Instead, the binding of HR-2 peptide DP178 to soluble HIV-1 Env 89.6 gp120 was monitored using two different ~~BIAcore~~ BIACORE binding assays, termed the “capture assay” and the “SA (streptavidin)-chip assay”.

Please replace the paragraph beginning at page 59, line 7, with the following rewritten paragraph:

When the precipitations were carried out in the presence of mAb A32 or sCD4, significant differences in DP178 binding were observed when compared to those observed in its absence. In the presence of A32 mAb, the means of the density of the bands in lane 3 were significantly higher than those in their absence (Figs. 29A and 29C, \*  $p < 0.005$ , lane 1 versus lane 3). This was also true when precipitates obtained in the presence of sCD4 were compared to those in their absence (Figs. 29B and 29C, \*\*  $p < 0.001$ , lane 1 versus 3). Therefore, the HR-2 peptide precipitation studies confirmed the observations using ~~BIAcore~~ BIACORE assays that both A32 mAb and sCD4 induced enhanced binding of HR-2 peptide DP178 to soluble gp120 Env proteins.

Before the Figures, insert the Sequence Listing submitted herewith.